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13. ABSTRACT (Maximum 200 Words)
Incidence rates of prostate cancer are higher in blacks than in any other racial group. Our laboratory is attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer in blacks. It is hypothesized that Africans may have genetically down-regulated their zinc absorption capacity; otherwise, they would absorb abnormally high levels of zinc, resulting in various serious neurodegenerative disorders. We hypothesized that people of African origin may have a lower capacity to absorb zinc when compared with other racial groups because of their inherent down-regulation of zinc transporters. This notion was tested by evaluating 58 prostate cancer tissues in 2 major racial groups (30 from whites and 28 from blacks) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from white people, the degree of expression of these 2 zinc receptors was high when compared with age-matched specimens obtained from blacks. Once these data are confirmed in larger groups, this finding could have significant application as a preventive maneuver for at least for some people. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

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Introduction

Why are African American men, after 10 to 20 generations of residing in the U.S., twice as likely to develop cancer of the prostate as Caucasian Americans, while South African Blacks are 10 to 30 times less likely to develop prostate cancer than their American distant cousins, and 2 to 10 times less likely than African Whites presumed to have a mixed European ancestry somewhat similar to that of White Americans? Are there any consistent differences in the expression of significant genes or proteins in the prostate cancers taken from African Americans versus those from White Americans? A study of the genes and proteins which influence the expression of any gene confirmed to be disparately expressed might lead to the identification of one or more environmental or living pattern factors worthy of epidemiological research for its potential relationship to the incidence or progression of prostate cancer in American Blacks, Whites, or other ethnic groups.

In this research project, it is our goal to test this hypothesis by analyzing prostate cancer tissues for the expression of mRNAs that code for various zinc transporter proteins. Our goals are to: 1) determine the expression levels of all three zinc *transporters (hZIP1, hZIP2* and *hZIP3*) in the neoplastic prostates from African Americans verses Whites by utilizing the quantitative reverse-transcriptase (RT) polymerase chain reaction PCR (RT-PCR), RT *in situ*-PCR, and immunocytochemistry methods, 2) measure the expression levels of all three zinc transporters (*hZIP1, hZIP2* and *hZIP3*) in the normal prostatic tissues from African Americans verses Whites, 3) measure the intracellular zinc levels and the location of zinc inside the various cell types that make up the cancer and normal prostate tissues, and examine in a few specimens the gene sequences of zinc transporters and their promoters, which presumably regulate the degree of expression of these genes, and 4) evaluate the zinc, testosterone and prolactin levels in the blood samples of over 2,000 individuals from all major races, especially in African American verses Whites. If a direct or environmental link between Zinc transport and prostate cancer can be established, then a special nutritional formula, medication, or other intervention might be especially designed to test the ability to decrease the incidence of this disease in African Americans. Such an intervention, if successful, might be useful for persons of all populations.

In the United States, prostate cancer is the most commonly diagnosed male cancer and the second leading cause of all male cancer deaths. African-Americans have the highest prostate cancer incidence rates in the world. Our laboratories are attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer, with special emphasis on the disproportionately high incidence rates in African-Americans. It is hypothesized that since Africa is a mineral-rich continent and the zinc levels in the water and diet are very high, Africans may have genetically down-regulated their zinc absorption capacity; otherwise they would absorb abnormally higher levels of zinc, which reportedly results in serious neurodegenerative and biochemical disorders. Therefore, individuals of African origin may have a lower capacity to absorb zinc due to their inherent down- regulation of zinc transporters when compared to other racial groups. Extensive research has shown that the low serum levels of zinc have been associated with the increased incidence of prostate cancer. Our laboratories have been collaborating with the Cleveland Clinic Foundation, the Medical College of Wisconsin, and the Medical Examiner's Office of the State of Maryland to determine the degrees of expression of various zinc transporters at the molecular level. Therefore, we have evaluated 58 prostate cancer tissue samples in 2 major racial groups (30 from Caucasians and 28 from African-Americans) for their ability to express two major human zinc transporters, hZIP1 and hZIP2. In all of the 30 prostate cancer specimens obtained from Caucasian individuals, the degree of expression of these two zinc receptors was higher when compared to age matched and the tumor grade level score matched specimens obtained from African-American patients. We also found significant down-regulation of these two zinc transporters in normal prostate tissues from African-American men as compared to age matched Caucasian men. When compared with normal prostate tissues, the expression levels of the zinc transporters were relatively lower in the neoplastic tissues from both racial groups tested. The loss of a unique capability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. However, there are several questions that need to be answered before a firm correlation can be established between the actual intracellular zinc levels in the prostate glands of various racial groups and the incidence rate of prostatic neoplasm. In addition, a causal relationship needs to be determined between the zinc levels in the prostate and the levels of expression of zinc transporters, in situ. Therefore, in order to answer these questions at the molecular and cellular levels, our goals are to examine the prostate tissues and sera from the corresponding patients and explore the following **Specific Aims**:

1) To determine the expression levels of all three zinc *transporters (hZIP1, hZIP2* and *hZIP3*) in the neoplastic prostates from African Americans verses Whites, by utilizing the quantitative reverse-transcriptase (RT) polymerase chain reaction PCR (RT-PCR) method, RT *in situ*-PCR, and immunocytochemistry.

2) To measure the expression levels of all three zinc transporters (*hZIP1, hZIP2* and *hZIP3*) in the normal prostate tissues from African Americans verses Whites.

3) To measure the blood zinc levels in about 2,000 individuals and compare the serum zinc levels in African-Americans, Africans, Caucasians, Asians and mixed racial groups. These studies will be performed at the Principal Investigator's site-Claflin University--a HBCU. For this purpose, the majority of the serum specimens will be obtained from the Medical University of South Carolina (MUSC). In addition to zinc levels, we will also measure the testosterone and prolactin levels in these groups.

4) In a separate but smaller group of individuals, autometallography and atomic absorption spectrophotometry will be used to measure intracellular zinc in various anatomic parts of the prostate tissues.

5) To determine which other factors, including the exposure to prolactin, testosterone, external zinc concentrations, and combinations of these three agents regulate the zinc transporters in the pre-established prostatic cell lines (i.e. PC-3 and La cell lines) and primary cell lines established from the prostate tumors from various racial groups. We believe that by establishing a link between the low intracellular transport capacity of zinc in the African-American population and development of prostate cancer, we may be able to design protocols which can increase intracellular zinc levels in the prostate gland. In addition, we hope to identify certain unique genes that may be selectively expressed or suppressed in certain racial groups. These studies may also shed some light on why men from all races develop prostate cancer in old age and how it is linked to intracellular zinc levels and serum zinc, testosterone, and prolactin levels.

PROGERESS IN THE FIRST 12 Months

AIMS 1-2:

To determine the expression levels of all three zinc *transporters (hZIP1, hZIP2* and *hZIP3*) in the neoplastic prostates from African Americans verses Whites, by utilizing the quantitative reverse-transcriptase (RT) polymerase chain reaction PCR (RT-PCR) method, RT *in situ*-PCR, and immunocytochemistry.

To measure the expression levels of all three zinc transporters (*hZIP1, hZIP2* and *hZIP3*) in the normal prostate tissues from African Americans verses Whites.

We are pleased to inform the agency that we have completed the AIMS #1-2 of the project and that an article is in press (galley proof is attached as Appendix 1). The main points of our data are described below.

ABSTRACT: As it is mentioned above that in the United States, prostate cancer is the most commonly diagnosed male cancer and the second leading cause of all male cancer deaths. Furthermore, incidence rates are higher in African Americans than in any other racial group. Our laboratory is attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer in African Americans. Because Africa is a mineral-rich continent, and the zinc levels in the water and diet are high, it is hypothesized that Africans may have genetically down-regulated their zinc absorption capacity; otherwise, they would absorb abnormally high levels of zinc, resulting in various serious neurodegenerative and biochemical disorders. It is therefore possible that people of African origin may have a lower capacity to absorb zinc when compared with other racial groups because of their inherent down-regulation of zinc transporters. This notion is further supported by our new initiatives in the areas of diabetes mellitus, hypertension, cardiovascular disease, and pancreatic cancer, incidence of which are higher in African Americans appears to be linked to zinc transporters. Extensive research has shown that low serum levels of zinc are associated with the increased incidence of prostate cancer. We have evaluated 58 prostate cancer tissues in 2 major racial groups (30 from whites and 28 from African Americans) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer

specimens obtained from white people, the degree of expression of these 2 zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients. We also found a significant down-regulation of these two zinc transporters in normal prostate tissues from African American men when compared with age-matched White men. The loss of the unique ability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. Our observation that the uptake of zinc may be different in racial groups is intriguing and relevant. Once these data are confirmed in larger groups, this finding could have significant application as a preventive maneuver for at least for some people. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

Details of the Results: The prostate contains high amounts of free zinc ions that are excreted into the seminal fluid. The extracellular and intracellular distribution of zinc ions in the rodent using highly specific autometallographical studies have shown that zinc accumulates primarily in the acinic lumen of the lateral lobes, whereas the dorsal lobe stains only faintly and the ventral lobe is void of grains (1,2). At the ultrastructural levels, the presence of zinc ions is confined to apical secretory vesicles and the epithelium of mainly the lateral lobes in both rodents and humans (1,3). Recently, Iguchi et al, using semiquantitative reversetranscription polymerase chain reactions (SQ-RT-PCRs), showed that the expression of zinc transporters (ZnT) in rats was very high in the lateral and dorsal prostate and much lower in the ventral prostate. In humans, it appears that the zinc ions are constantly secreted from the epithelial cells into both the acinic lumen and the intercellular canaliculi (5). Prostate secretory epithelial cells have the unique function and capability of accumulating extremely high intracellular levels of zinc (2–5). One of the effects of this accumulation is the inhibition of cell growth, partly because of an increase in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial apoptogenesis (6). Prolactin and testosterone regulate zinc accumulation in the prostate; however, little information is available concerning the mechanisms associated with zinc accumulation and its regulation in prostate epithelial cells (7,8). By using the human malignant prostate cell lines LNCaP and PC-3, Costello et al (7) have shown that the zinc accumulation in both cell types is stimulated by physiologic concentrations of prolactin and testosterone. Their studies reveal that these cells possess the ability to uptake zinc rapidly, indicative of the presence of a plasma membrane high-affinity zinc transporter, possibly by the regulation of the

ZIP-type zinc transporter gene expression (7,8). Kinetic studies demonstrate that the rapid uptake of zinc is effective under physiologic conditions that reflect the total and mobile zinc levels in circulation (8) Correspondingly, genetic studies demonstrate the expression of a ZIP family zinc uptake transporter in both LNCaP and PC-3 cells (8). Some of these zinc-accumulating characteristics are found to be specific for prostate cells. These studies support the concept that prostate cells express a unique hormone-responsive, plasma membrane-associated, rapid zinc uptake transporter gene that is associated with their unique ability to accumulate high zinc levels (9–11). In the United States, the incidence of prostate cancer is significantly higher in African Americans than in White people or Asian Americans (12–19). We hypothesized that because Africa is a mineral-rich continent and zinc levels are relatively high in the water and diet, abnormally high amounts of zinc in the blood may result in various neurologic and metabolic abnormalities. The zinc absorption and transport systems are genetically down regulated in the African populations. The phenomenon may be similar to sickle cell anemia, in which a single mutation has provided the survival advantages against the ravages of the fatal form of malaria (20). We hypothesize that when African people entered North America mostly during the slave trade, they encountered an environmental problem: in North America, the zinc levels are relatively low, and the native populations (the American Indians) and other races who migrated from Europe and Asia have a higher capacity to transport zinc to various organs, especially to the prostate gland, in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in the White and Black populations (21). Here the situation is reversed. The White population is unable to up-regulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect them from damaging solar ultraviolet (UV) light. Squamous cell carcinoma is the most common tumor of sunexposed epithelium in White populations (21). Therefore, just as White people carry an evolutionary disadvantage against the solar UV rays outside of their ancestral low-UV light environment, the low absorption capacity of zinc has created a disadvantage in the peoples of African descent who have migrated outside Africa. If this is the case, long-term low serum concentrations of zinc deprive the prostate gland of its essential source of vital trace mineral ingredients, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the idea that low levels of zinc contribute to the high incidence of prostate cancer (1–10) In this research study, it was our goal to test this hypothesis by analyzing the prostate tissues for their ability to express 2 major zinc transporters responsible for the accumulation of zinc in the prostate glands.

For this purpose, we used a highly sensitive RT-in situ-PCR method to compare the relative levels of expression of the 2 zinc transporters, *hZIP1* and *hZIP2,* in 2 racial groups in the United States (White and African American).

African Americans have the highest prostate cancer incidence rate in the world (13–18,25) On a global level, the rates of incidence are low in Asian and African men, low to moderate in White men, and high in African American men. 13,25 Using data collected between 1988 and 1992, Wingo et al1 reported that African Americans have a 35% higher incidence rate and a 223% higher mortality rate from prostate cancer when compared with Whites.

The differences in the incidence and mortality between African Americans and Whites are attributed to screening, environmental, and biological factors (16,17). When compared with White controls, Black men present at a younger age with a higher grade and stage of the disease for their age, and with a greater delay in diagnosis (18,19). Whether the pathogenesis of prostate cancer is different in African American men compared with White men remains unanswered. Whittemore et al (19,25) note that African American men appear to have a larger volume of "latent prostate cancer." These investigators believe that larger-volume latent carcinomas are those that progress to become clinically evident at a faster rate, suggesting that the events that account for racial differences in prostate cancer incidences may occur very early in cell transformation, and thus may be genetically controlled. We have hypothesized that the major reason for the high incidence of prostate cancer in African Americans may be their inherent inability to absorb or transport normal amounts of zinc from the Northern American environment, in which there is a relatively low concentration of zinc in the diet. We have further hypothesized that zinc absorption and transport systems are genetically down-regulated in some of the African populations. Such natural selection may occur because of the serious adverse effects on the nervous system caused by high zinc levels (26–28). However, when African people entered North America, mostly during the slave trade, they may have encountered an environmental disadvantage because of their inherent down-regulation of zinc transporters. On this continent, the zinc levels are relatively low, and the native populations (the American Indians) and other races who migrated from low zinc areas, ie, Europe and Asia, have a higher capacity to absorb zinc and are able to transport it to the other organs in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in white and nonwhite populations.

The white population is unable to upregulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect themselves from the damaging solar UV light of wavelengths between 290 and 320 nm. Skin cancer is the most common type of cancer in the United States; more than 600,000 cases of skin cancer are reported each year in this country in the white population. Squamous and basal cell carcinomas are the most common tumors of sun-exposed skin areas in this group (21). Just as white people carry an evolutionary disadvantage against the solar UV light outside their low UV light ancestral environment, the low absorption capacity of zinc has created a disadvantage in people of African descent when they migrate outside Africa. Long-term low serum concentration of zinc deprives the prostate gland of its essential source of a vital trace mineral ingredient, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the association of low levels of zinc with prostate neoplasia (3–6). Zinc is an essential nutrient to all organisms because it is a required catalytic or structural cofactor for hundreds of zinc-dependent enzymes and other proteins such as transcription factors. An example of the effect of the low serum levels of zinc can be seen in the animal model of the *lethal milk* mouse mutant (29). ZnT4 is deficient in the *lethal milk* mouse mutant, in which pups of any genotype suckled on homozygous *lethal milk* mothers die of zinc deficiency before weaning. The zinc level in the milk of homozygous *lethal milk* animals is approximately 50% than that of normal animals, demonstrating that ZnT4 plays a crucial role in development (29). Various reports suggest that regardless of race and geographic location, the etiology of certain prostate carcinomas may be linked to zinc transporters. Because the expression of zinc transporters also appears to be regulated by prolactin and testosterone, an age-related increase in the incidence rate of this malignancy may also be indirectly linked to the zinc uptake by the prostate gland (8–11). Members of the *ZIP* family are found in all cellular life forms, including archaebacteria, eubacteria, and eukaryotes (10,30–34). There are currently approximately 85 members reported in the sequence databases. These fall into 4 subfamilies based on their amino acid similarities.10 Most members are predicted to have 8 transmembrane domains and share a predicted topology where the amino and carboxyl termini are extracytoplasmic. There are 12 known ZIP members in the human genome.31 Three of the human proteins, *hZIP1, hZIP2*, and *hZIP3*, are very closely related to the fungal and plant proteins known to be zinc uptake transporters. *hZIP2* expression has been detected only in prostate (31) and uterine (32) epithelial cells, suggesting that this protein plays a very specialized tissue-specific function. On the other hand, hZIP1 is expressed in all 24 human tissues examined so far.33 Gaither and Eide (10,11,31) have

sequenced and characterized the *hZIP2* gene, a human zinc transporter identified by its similarity to zinc transporters recently characterized in fungi and plants. *hZIP2* is a member of the *ZIP* family of eukaryotic metal ion transporters that includes 2 other human genes, *hZIP1* and *hZIP3*, and the genes in mice and rats (10,11). The human genome contains at least 3 *ZIP* family members. The current hypothesis is that these genes encode zinc uptake transporters (10,11) We can gain some insight into *ZIP* function in humans by considering the tissues in which these proteins are expressed. Repeated attempts by Gaither and Eide11 to detect *hZlP2* mRNA on Northern blots of poly (A)+ RNAs derived from different human tissues and cultured cell lines failed to produce positive results. It appears that the *hZIP2* transporter gene is normally expressed at low levels and in specific cell types, and that a more sensitive detection method is required. We also attempted to quantitate *hZIP2* mRNA by Northern blots; however, several attempts were not productive. Therefore, we decided to use highly sensitive RT-in situ-PCR and SQ-RT-PCR methods. Gaither and Eide10 isolated only 4 *hZIP2*-expressed sequence tag clones found only in prostate and uterine cDNA libraries. The observation that these particular tissues express *hZIP2* may be instructive in that cells of the prostate contain the highest zinc level of any soft tissue in the body. Any potential down-regulation in this transporter may play a pivotal role in the pathogenesis of prostate cancer. Thus, it appears that the expression of *hZIP2* in prostate and uterine tissues may help meet their particular needs of zinc metabolism. In contrast, the low affinity *hZIP1* and *hZIP3* have been cloned as expressed sequence tags from a large number of different tissues, indicating that these genes are widely expressed and may play general housekeeping roles (10). Therefore, observed zinc transporter expression may be associated with the great need for zinc involved in the normal processing of the prostate gland functions, a lack of which may have caused the molecular injury resulting in the development of prostate cancer (7–11). Low serum levels of zinc have been associated with the increased incidence of prostate cancer (7–12). Previously, Costello et al (8,9) have shown that *hZIP1* is expressed in PC-3 cells, and that a zinc update actively upregulated by testosterone and prolactin treatment. Furthermore, *hZIP1* expression was regulated by zinc availability. Therefore, when PC-3 cells were exposed to high zinc, *hZIP1* mRNA levels were down-regulated. The molecular mechanisms by which low zinc levels contribute to the development of neoplasia are still obscure, and limited data are available. Costello et al (9) have shown that long-term cellular zinc deficiency leads to an increase in cell proliferation partly because of a reduction in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial

apoptogenesis, indicating a physiologic effect of zinc in the regulation of prostate cell growth. Thus, in prostate cancer, 2 themes emerge from the analyses of zinc transporter expression in vivo: (1) the down-regulation of zinc transporters by either genetic inheritance (African descent) or through aging (related to the modulations in the testosterone/prolactin levels or gene expressions acquired with old age) leads to the low accumulation of zinc in the prostate tissues, (12–19) and (2) the loss of the unique capability to retain normal intracellular levels of zinc caused by either the increased export or low import of zinc may be an important factor in the development and progression of malignant prostate cells (1–5,10,29–35). From our data, it appears that the lowest degree of the expression of zinc transporters, *hZIP1* and *hZIP1,* is localized in the areas that exhibit neoplastic lesions, and is less dominant in the areas that are healthy-appearing. Our observation that there are differences in the zinc transport in different racial groups has great significance for prevention. If a role of zinc transporters is clearly established, then a zinc supplementation could be helpful in at least some people. Understanding the molecular events in the pathogenesis of prostate cancer is critical to the evaluation of the natural history of prostate cancer in humans, especially in various racial groups (34–38).

AIM # 3: The Aim of this project was to measure the blood zinc levels in about 2,000 individuals and compare the serum zinc levels in the African-Americans, Africans, Caucasians, Asians, and the mixed race groups. These studies will be performed at the Principal Investigator's site-Claflin University- an HBCU. For this purpose, the majority of the serum specimens will be obtained from the Medical University of South Carolina (MUSC). In addition to zinc levels, we will also measure the testosterone and prolactin levels in these groups.

This portion of the study is in progress and the PI has started to collect the well defined specimens from two local clinics in Orangeburg. In addition, we are in the process of getting an Internal Review Board (IRB) approval from the Claflin University IRB so we can also collect blood specimens from the students who consent to it. Claflin University has a diverse student population from various racial groups that would provide a rich source of information. Our collaborator at MUSC, Dr. Tim Garvey, is no longer at this institute and moved to the University of Alabama at Burmingham (UAB). We are also attempting to acquire specimens from him after he settles in at UAB, and acquire the approval from IRB from UAB.

Meanwhile, the PI is training two students on various methods so once a sufficient number of specimens is received they can run the assays in batches with the appropreate standard curves.

AIM # 4: In a separate but smaller group of individuals, autometallography and atomic absorption spectrophotometry will be used to measure intracellular zinc in various anatomic parts of the prostate tissues.

We have collected over 50 specimens to run this assay, and currently we are setting up this assay.

AIM # 5: To determine which other factors, including exposure to prolactin, testosterone, external zinc concentrations, the combinations of these three agents, and other factors regulate the zinc transporters in the pre-established prostatic cell lines (i.e. PC-3 and La cell lines) and primary cell lines established from the prostatic tumors from various racial groups. We believe that by establishing a link between the low intracellular transport capacity of zinc in the African-American population and the development of prostate cancer, we may be able to design protocols that can increase intracellular zinc levels in the prostate gland. In addition, we hope to identify certain unique genes that may be selectively expressed or suppressed in certain racial groups. These studies may also shed some light on why men from all races develop prostate cancer in old age and how it is linked to intracellular zinc levels and serum zinc, testosterone, and prolactin levels.

The PI has trained two undergraduate students to perform cell cultures on various prostate cell lines. These students have learned the RT-in situ PCR method so they can perform zinc transporter expression assays in vitro. We hope to make significant progress in this Specific Aim in a very short time.

KEY RESEARCH ACCOMPLISHMENTS

- ❖ We began with a crucial question: Why are African American men, after 10 to 20 generations in the U.S., twice as likely to develop cancer of the prostate as

Caucasian Americans, while South African Blacks are 10 to 30 times less likely to develop prostate cancer than their American distant cousins and 2 to 10 times less likely than African Whites, who may be presumed to have a mixed European ancestry somewhat similar to that of White Americans?

- In order to answer this question in a most definitive fashion, we have divided our possible answers into various categories. The first and foremost of the question was to determine if there are any consistent differences in the expression of significant genes or proteins in the prostate cancers taken from African Americans versus those from White Americans. A study of the genes and proteins which influence the expression of any gene confirmed to be disparately expressed might lead to the identification of one or more environmental or living pattern factor worthy of epidemiological research for its potential relationship to the incidence or progression of prostate cancer in African Americans, Whites, or other groups. For this purpose we chose to evaluate the relative degree of expression of human zinc transporters crucial for retaining the zinc into the prostate.

- We have evaluated 58 prostate cancer tissues in 2 major racial groups (30 from whites and 28 from African Americans) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from White people, the degree of expression of these 2 zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients.

- We also found a significant down-regulation of these two zinc transporters in normal prostate tissues from African American men when compared with age-matched White men.

- We have began to set up the highest state of the art methods to determine the intracellular levels of zinc in the 50 specimens collected until this date. Our goal is to measure the intracellular zinc levels and the location of zinc inside the various cell types that make up the cancer and normal prostate tissues. Two undergraduate minority students have completed their preliminary training in the PI's laboratory, and we will analyze the patients' tissue specimens within a few weeks.

- We already have trained two additional undergraduate minority students to carry out tissue culture methods on well-defined prostate cancer cell lines. These cell lines will be analyzed for the relative expression of zinc transporters before and after the exposuere to various concentrations of zinc, testosterone, and prolactin.

- The blood samples that were supposed to come from MUSC most probably will not be available due to the move of our collaborator to UAB. We have initiated collaborations with two local clinics in Orangeburg and at out own institute to collect the specimens. We will be able to do so and would be able to collect over 2,000 blood specimens from all major races to evaluate concentrations of zinc, testosterone, and prolactin in African Americans versus Whites.

- We are certain that there is a direct link between zinc transport and prostate cancer. If a strong link is established between the environment, genes, and diet, then a special nutritional formula, medication, or other intervention might be especially designed to test the ability to decrease the incidence of this disease in African Americans. Such an intervention, if successful, might be useful for the persons of all populations.

REPORTABLE OUTCOMES:

Manuscripts: A manuscript is in print and attached as Appendix 1. This article will appear in a peer-reviewed journal before the end of this year.

- Irum Rishi, J.A. Abbasi, R. Bullard-Dillard A. Balla, R. Tubbs, **O. Bagasra**. 2003. Down-regulation of *hZIP*1 and *hZIP2* zinc transporters in the prostate cancer tissues from African descent as compared to White men (In Press: **Applied Immunochemistry and Molecular Morphology**).
- Bagasra, O. E. Jones, S. Collins, et al. **Role of Zinc Transporters in Various African American Diseases (In preparation).**

Abstracts: The following Abstract has resulted from this award;

- Bagasra, O, et al. 2002. Prostate cancer in African American men is associated with the down regulation of zinc transporters. 93rd Annual Meeting of AACR Abst# LB 155.

Presentation: The PI presented data on prostate cancer at the following locations:

- Bagasra, O. New Frontiers in Morphology. 10th International Conference of molecular morphology. Oct 5-8, 2002. Santa Fe, NM.
- Bagasra, O. A New Revolution in Vaccinology. Invited speaker at Touro University, Merre Island, CA. May 19, 2003.
- Bagasra, O. A New Idea in Prostate Cancer Prevention. Invited speaker at San Francisco State University. San Franciasco, CA. May 22, 2003.

Patents and licenses applied for and/or issued: None

Degrees obtained that are supported by this award:

Two minority students worked on this project as part of the requirement of their undergraduate degree:

- Tiffany Brown
- Melodie Harrison

Development of cell lines, tissue or serum repositories; infomatics such as databases and animal models, etc. None

Funding applied for based on work supported by this award:

An application was submitted to NCI based on the preliminary result from this award and would be funded. The details are as follows;

NCI: 08/01/2003 to 04/31/06 "Training grant for Claflin University Students"
In collaboration with USC Cancer Center
$566,035/yr with USC Cancer Center, P.I.: O. Bagasra

Employment or research opportunities applied for and/or received based on experience/training supported by this award. YES!

NCI: 08/01/2003 to 04/31/06 "Training grant for Claflin University Students"
In collaboration with USC Cancer Center
$566,035/yr with USC Cancer Center, P.I.: O. Bagasra

CONCLUSIONS: We started our project to address a very important question concerning why African American men, after 10 to 20 generations in the U.S., twice as likely to develop cancer of the prostate as Caucasian Americans, while South African Blacks are 10 to 30 times less likely to develop prostatic cancer than their American distant cousins and 2 to 10 times less likely than African Whites, who may be presumed to have a mixed European ancestry somewhat similar to that of White Americans. Are there any consistent differences in the expression of significant genes or proteins in the prostate cancers taken from African Americans versus those of White Americans? A study of the genes and proteins which influence the expression of any gene confirmed to be disparately expressed might lead to the identification of one or more environmental or living pattern factors worthy of epidemiological research for its potential relationship to the incidence or progression of prostate cancer in American Blacks, Whites, or other groups.

We have concluded that there are significant differences between Whites and African Americans with regards to the degree of expression of two zinc transporters that are involved in importing zinc from the outside into the prostate glands. There are many additional assays that need to be performed. Once these data are confirmed in larger groups, this finding could have a significant application as a preventive maneuver at least in African Americans. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

Also, African Americans disproportionately suffer from various diseases in the US. Many of these diseases include hypertension, lupus, cardiovascular disease, diabetes mellitus, and cancers of the prostate and pancreas. A number of risk factors such as smoking, a high fat diet, little physical activity, stress, and meager access to health care have been the subject of numerous investigations. However, the factor of the interaction between genetics and the environment has received very little attention in the basic

scientific community.

According to numerous epidemiological data in the 21st century, patients suffering from DM will increase more than in the 20th century. For those reasons, the creation and development of a new class of pharmaceuticals for the treatment of DM in the 21st century is extremely desirable. In the last half of the 20th century, investigations between the relationships among diseases and micronutrients, such as iron, copper, zinc, and selenium, have been numerous.

Our laboratory is investigating the potential role of zinc transporters in the pathogenesis of many illnesses that disproportionately affect the African American community.

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Prostate Cancer in African American Men Is Associated With Downregulation of Zinc Transporters

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In the United States, prostate cancer is the most commonly diagnosed male cancer and the second leading cause of all male cancer deaths. Furthermore, incidence rates are higher in African Americans than in any other racial group. Our laboratory is attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer in African Americans. Because Africa is a mineral-rich continent, and the zinc levels in the water and diet are high, it is hypothesized that Africans may have genetically downregulated their zinc absorption capacity; otherwise, they would absorb abnormally high levels of zinc, resulting in various serious neurodegenerative and biochemical disorders. It is therefore possible that people of African origin may have a lower capacity to absorb zinc when compared with other racial groups because of their inherent downregulation of zinc transporters. Extensive research has shown that low serum levels of zinc are associated with the increased incidence of prostate cancer. We have evaluated 58 prostate cancer tissues in 2 major racial groups (30 AU4 from whites and 28 from African Americans) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from white people, the degree of expression of these 2 zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients. We also found a significant downregulation of these 2 zinc transporters in normal prostate tissues from African American men when compared with age-matched white men. The loss of the unique ability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. Our observation that the uptake of zinc may be different in racial groups is intriguing and relevant. Once these data are confirmed in larger groups, this finding could have significant application as a preventive maneuver for at least for some people. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

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The prostate contains high amounts of free zinc ions that are excreted into the seminal fluid. The extracellular and intracellular distribution of zinc ions in the rodent using highly specific autometallographical studies have shown that zinc accumulates primarily in the acinic lumen of the lateral lobes, whereas the dorsal lobe stains only faintly and the ventral lobe is void of grains.[1,2] At the ultrastructural levels, the presence of zinc ions is confined to apical secretory vesicles and the epithelium of mainly the lateral lobes in both rodents and humans.[1,3] Recently, Iguchi et al,[4] using semiquantitative reverse-transcription polymerase chain reactions (SQ-RT-PCRs), showed that the expression of zinc transporter (ZnT) 2 in rats was very high in the lateral and dorsal prostate and much lower in the ventral prostate. In humans, it appears that zinc ions are constantly secreted from the epithelial cells into both the acinic lumen and the intercellular canaliculi.[5] Prostate secretory epithelial cells have the unique function and capability of accumulating extremely high intracellular levels of zinc.[2–5] One of the effects of this accumulation is the inhibition of cell growth, partly because of an increase in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial apoptogenesis.[6] Prolactin and testosterone regulate zinc accumulation in the prostate; however, little information is available concerning the mechanisms associated with zinc accumulation and its regulation in prostate epithelial cells.[7,8] By using the human malignant prostate cell lines LNCaP and AU5 PC-3, Costello et al[7] have shown that the zinc accumulation in both cell types is stimulated by physiologic concentrations of prolactin and testosterone. Their studies reveal that these cells possess the ability to uptake zinc rapidly, indicative of the presence of a plasma membrane high-affinity zinc transporter, possibly by the regulation of the ZIP-type zinc transporter gene expression.[7,8] Kinetic studies demonstrate that the rapid uptake of zinc is effective under physiologic conditions that reflect the

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total and mobile zinc levels in circulation.[8] Correspondingly, genetic studies demonstrate the expression of a ZIP family zinc uptake transporter in both LNCaP and PC-3 cells.[8] Some of these zinc-accumulating characteristics are found to be specific for prostate cells. These studies support the concept that prostate cells express a unique hormone-responsive, plasma membrane-associated, rapid zinc uptake transporter gene that is associated with their unique ability to accumulate high zinc levels.[9–11]

In the United States, the incidence of prostate cancer is significantly higher in African Americans than in white people or Asian Americans.[12–19] We hypothesized that because Africa is a mineral-rich continent and zinc levels are relatively high in the water and diet, abnormally high amounts of zinc in the blood may result in various neurologic and metabolic abnormalities. The zinc absorption and transport systems are genetically downregulated in AU6 the African populations. The phenomenon may be similar to sickle cell anemia, in which a single mutation has provided the survival advantages against the ravages of the fatal form of malaria.[20] We hypothesize that when African people entered North America, mostly during the slave trade, they encountered an environmental problem: in North America, the zinc levels are relatively low, and the native populations (the American Indians) and other races who migrated from Europe and Asia have a higher capacity to transport zinc to various organs, especially to the prostate gland, in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in the white and black populations.[21] Here the situation is reversed. The white population is unable to upregulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect them from damaging solar ultraviolet (UV) light. Squamous cell carcinoma is the most common tumor of sun-exposed epithelium in white populations.[21] Therefore, just as white people carry an evolutionary disadvantage against the solar UV rays outside of their ancestral low-UV light environment, the low absorption capacity of zinc has created a disadvantage in the peoples of African descent who have migrated outside Africa. If this is the case, long-term low serum concentrations of zinc deprive the prostate gland of its essential source of vital trace mineral ingredients, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the idea that low levels of zinc contribute to the high incidence of prostate cancer.[1–10]

In this research study, it was our goal to test this hypothesis by analyzing the prostate tissues for their ability to express 2 major zinc transporters responsible for accumulation of zinc in the prostate glands. For this pur- AU7 pose, we used a highly sensitive RT-in situ-PCR method to compare the relative levels of expression of the 2 zinc transporters, *hZIP1* and *hZIP2*, in 2 racial groups in the United States (white and African American).

METHODS AND MATERIALS

Human Subjects and Study Protocol

Archival, formalin-fixed, paraffin-embedded specimens of primary prostate carcinoma were retrieved from the files at the Department of Pathology of the Medical College of Wisconsin. Similarly, fixed tissues from radical prostatectomy specimens were obtained from the Cleveland Clinic Foundation, according to the approved protocols of the respective institutes. Normal prostate tissues were autopsy specimens obtained from the State of Maryland Medical Examiner's Office at Baltimore, Maryland.

Quantitative Reverse-Transcription Polymerase Chain Reaction Zinc Transporters

The total mRNAs were harvested from the deparaffinized tissues as described previously.[22–24] The RNA preparations were used to quantitate the levels of zinc transporters, and the relative levels of expression were visualized according to each racial subgroup.

Semiquantitative Reverse-Transcription–initiated Polymerase Chain Reaction AU8

The details of the SQ-RT-PCR have been described previously.[22–24] The major advantage of this protocol that allows the relative quantitation of each of the specific RNA species in the samples is the use of standard curves of in vitro transcribed mRNAs of *hZIP2*. Plasmids containing full-length *hZIP2* and pCMV-hZIP2 were AU9 kindly provided by Dr. David J. Eide of the Department of Nutritional Sciences, University of Missouri. This clone was used to develop a standard curve to semiquantitate the relative degree of the expression of *hZIP2*. The full-length *hZIP2* shows a significant homology to the members of the *ZIP* family, including *hZIP1*. Therefore, we were able to design primer pairs that could amplify the conserved sequences of hZIP1 and hZIP2 by multiplex polymerase chain reaction. The concentrations of the mRNAs were measured spectrophotometrically, and the relative copy numbers of mRNAs present in 1 μL of solution were calculated using the molecular weight of the transcript and the Avogadro number. Relative numbers of each *ZIP* were derived by SQ-RT-PCR and gen- AU10 erated by a serial 2-fold dilution of *pCMV-hZIP2* plasmid DNA. In the linear amplification range of these curves, the copies of in vitro transcribed mRNAs were plotted against the relative size of the amplified bands of the amplified fragments of the 2 *hZIPs*. Using these dilution curves of the plasmid (performed in duplicate), the relative number of the spliced mRNAs for *hZIP1* and *hZIP2* were calculated. All samples were tested in at least 3 independent experiments. As a control, we performed quantitative RT-PCR of β-actin, as we described previously.[22–24]

AU11 **Reverse-Transcription–initiated in Situ Polymerase Chain Reaction**

Paraffin sections from 58 prostate biopsies of men with clinical histories of prostate cancer, and 4 from autopsy specimens of people with normal glands who died of automobile accidents, were processed for RT-in situ-PCR. Briefly, paraffin-embedded tissue sections of the specimens were received from each of our collaborators in a blinded fashion. All the reagents were pre- AU12 pared in RNase-free reagents. Therefore, all the slides were deparaffinized with sequential washings with EM-grade xylene, absolute alcohol, 95% ethanol, and 70% ethanol for 5 minutes each, then washed twice in a 1× phosphate-buffered saline (PBS). After deparaffinization, these slides were further fixed in a Streck fixative (STF; Streck Labs, Inc, Omaha, NE) for 2 hours. Incubating slides in 3× PBS for 10 minutes inactivated STF. The slides were washed twice in 1× PBS. These slides were treated with proteinase K (6 µg/mL) at room temperature for 22 minutes. Proteinase K was inactivated by incubating slides on a heat block at 95°C for 5 minutes. To perform the amplification of mRNA sequences for *hZIP1 and hZIP2,* we used multiply spliced sequences that flank the junctions of 2 exon splice sites. Because these RNA-specific primers will not amplify the genomic DNA template, one can perform the amplification of AU13 multiple mRNAs simultaneously. The following primer pairs were used: sense 5′-ACCAGACAAGGAC-TTCA-ATTAC-3′ and antisense 5′- GAGGACTAAAGCTGA-AAACATC-3′ for *hZIP1*, and sense 5′-GAATCACAG-ATTCAGAAGTTCA-3′ and antisense 5′-CTCTCCAT-AGGGATACTC CATA-3′ for *hZIP2*. The amplification of β-actin mRNA was performed by using a pair of primers: 5′-ATCTGGCACCTTCTACAATGAGCTGCCG-3 AU14 and 5′-CGTCATACTCCTGATTGCTGATCCACA-CATCTGC-3′. The *hZIP2* gave a 102-bp product and AU15 *hZIP1* gave a 189-bp product. The β-actin pair yielded 838-bp amplicons.[22–24] To amplify, we used the *rTth* enzyme, which has both the RT and polymerase function.[23] The amplification cocktail contained the pair of primers at 100 pM each in 50 mM Tris pH 8.3, 8.5 mM $MgCl_2$, 10 mM $MnCl_2$, 40 mM KCL, 1 mM dithiothreitol, 10× transcription buffer, 10× chelating buffer, 5 U AU16 *rTth* enzyme, and 200 mM of each deoxyribonucleoside triphosphate. Twenty µL RT cocktail was added to each slide. The slides were then sealed with slide frame sealer and inserted into the slide slots of a thermocycler spe- AU17 cially designed for in situ PCR (MJR-Twin Tower, PTC 200). The 2 cycles were programed for 30 minutes at 62°C, the 94°C for 2 minutes (for cDNA step), and then AU18 cDNAs were amplified for 30 cycles at 92°C/55°C/72°C.

AU19 Hybridizations were performed with either a Cy3 or an FITC oligonucleotide probe for the *hZIP1* and *hZIP2* sequences (oligonucleotide: 5′-CAGCAAGTGAGAGA-AATTCTTCTGGTGATGCTGATTCAGCTC-3′ and 5′-CTTAGAATTTCAGTGGAGTCTTTTTCCT-CTTGCAGTTTAAAGCAAAAGTC-3′). Hybridizations were performed in a buffer containing 50% formaldehyde, 10 mM dithiothreitol, 2× SSC, 100 µg/mL AU20 fragmented salmon sperm DNA, 2% bovine serum albumin, 1 mg/mL *Escherichia coli* tRNA, and 20 pmol probes at 95°C for 2 minutes, then 40°C for 18 hours. These tissue sections were then washed to remove unbound probes and viewed under UV epifluorescence microscopy after the cells were washed. To preserve the intensity of the hybridized probes, the tissues were not counter-stained. Parallel hematoxylin and eosin-stained slides were used to identify various histologic cell types in the tissue sections. Microscopic examination usually reveals cytoplasmic staining for mRNA versus nuclear staining for DNA.[22–24] Cell enumeration was performed on coded slides by at least 2 pathologists.

RESULTS

Degree of hZIP1 and hZIP2 Expression in the Malignant Prostate Tissues From White and African American Men

We evaluated the *hZIP1* and *hZIP2* expression, the 2 major zinc transporters, by simultaneously performing a multiplex RT-*in situ*-PCR. We evaluated 58 prostate cancer specimens in a blinded manner for their level of expression of these 2 zinc transporters. The majority of the specimens were from patients who had a 3+3 or 3+4 Gleason score. Upon unlocking the blinded codes, all the specimens from the white men exhibited a significantly higher degree of expression of the 2 zinc transporters than the majority of the specimens from the African Americans. Therefore, all 30 specimens from the white men's prostate biopsies exhibited a modest degree of expression of both of the zinc transporters, whereas 26 of 28 prostate specimens from the African Americans exhibited a low or very low expression of both the zinc transporters. In 1 of the other 2 specimens from African Americans, the prostate sections exhibited high expression of *hZIP1* and low expression of *hZIP2*, whereas in the second case, a reverse pattern of expression was observed. In Figure 1, we have shown representative mi- F1 crophotographs of 8 prostate intraepithelial lesions from age-matched specimens, 4 from each racial group.

Figure 2 shows high-grade prostate carcinomas from 6 F2 age-matched specimens, 3 from each racial group. As it is evident in Figures 1 and 2, the degree of expression of both zinc transporters is significantly higher in the prostate tissues from white men than in the age-matched lesions from the African Americans. The coexpression of the zinc transporters *hZIP1* and *hZIP2* in the neoplastic lesions and areas surrounding the tumor lesion showed a wide variation in the expression of these zinc transporters in all tissues from both races. However, with the

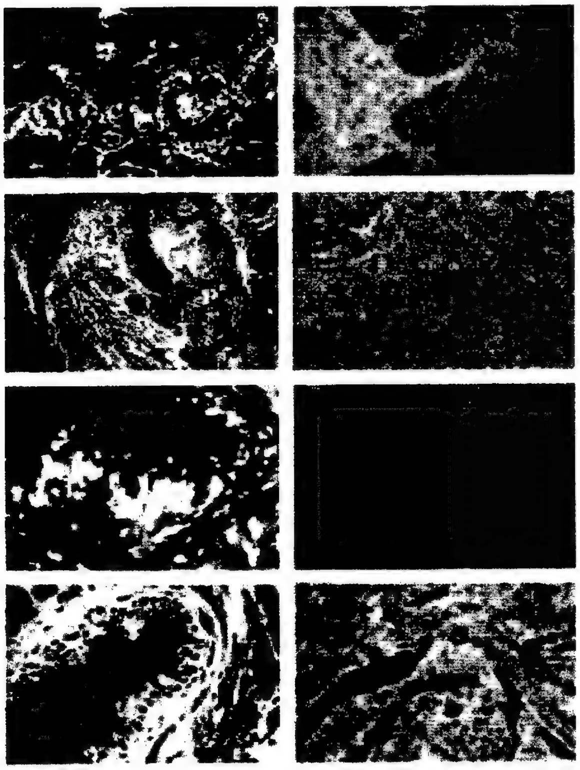


FIGURE 1. Representative photomicrographs showing the expression of 2 zinc transporters in intraepithelial lesions, *hZIP1* and *hZIP2*, by multiplex RT-in situ-PCR. Four intraepithelial neoplastic lesions from white men (left) and age-matched specimens from African American men (right) are shown. The coexpression of the zinc transporters, *hZIP1* (red) and *hZIP2* (green), the neoplastic lesions, and areas surrounding the tumor lesion exhibit a wide variation in the expression of these zinc transporters in the intraepithelial neoplastic tissues of the white group. In African Americans, the prostate cancer from the tumors and surrounding areas exhibited a markedly decreased expression of the zinc transporters compared with the tumors from the white patients.

exception of 2 cases, we observed a consistent decreased degree of the expression in prostate tissues from African Americans over that seen in their counterparts, regardless of their tumor grade. The prostate cancer from the African Americans' tumors and surrounding areas exhibited a markedly decreased expression of zinc transporters compared with the tumors from the white patients. Of note, in all cases, the expressions of both the zinc transporters were visibly lower in the neoplastic areas compared with the surrounding normal-appearing areas. This finding is consistent with the data indicating that overall, the zinc levels are lower in the malignant portions of the prostate gland.[3]

We also performed SQ-RT-PCR analyses in the mRNAs isolated from the prostate tissues of the 8 men shown in Figure 1 for *hZIP1,* and also from 6 people for hZIP2, shown in Figure 2. As shown in Figure 3, the relative expression in the specimens from African Americans of both zinc transporters, *hZIP1 and hZIP2,* were significantly lower when compared with their age-matched counterparts.

To demonstrate that mRNAs were not degraded in the paraffinized prostate specimens, we analyzed the presence and integrity of β-actin mRNAs by RT-PCR. RT-PCR for β-actin was performed on all the specimens. As shown in Figure 3, 8 specimens evaluated for *hZIP1* also had intact mRNAs for β-actin, which clearly demonstrate the integrity of mRNAs in the specimens we analyzed for the ZIP transporters. More importantly, there was no significant difference in the degree of amplification between the specimens isolated from white men and African Americans. These analyses validated 2 important points: (1) the mRNAs we isolated were intact, and (2) the differences we observed in the expressions of the

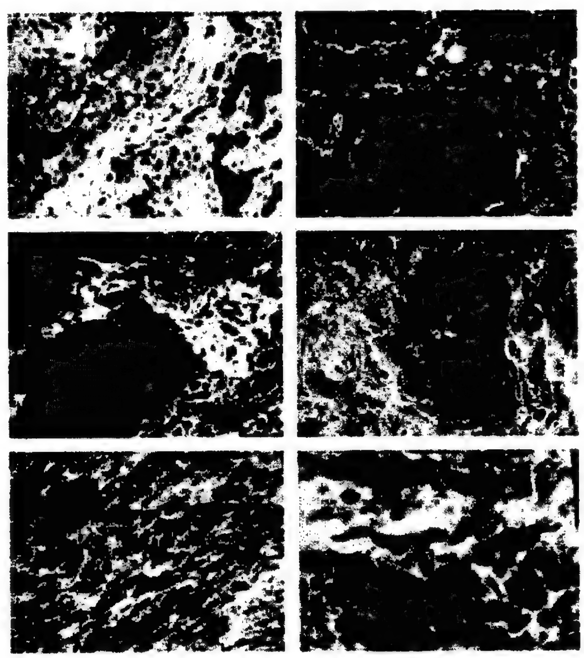


FIGURE 2. Representative photomicrographs showing the expression of the 2 zinc transporters in high-grade tumors, *hZIP1* and *hZIP2*, by multiplex RT-in situ-PCR. Shown are 6 intraepithelial neoplastic lesions, age-matched specimens, 3 from each racial group: white (left) and African American men (right). In African Americans, the prostate cancer from the tumors and surrounding areas exhibited a marked decrease in the expression of the zinc transporters compared with the tumors from the white patients.

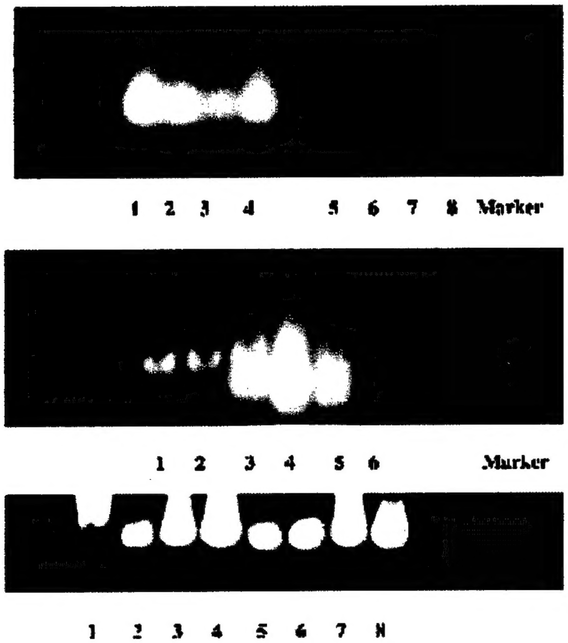


FIGURE 3. Semiquantitation of spliced mRNAs of *hZIP1* and *hZIP2* zinc transporters by QS-RT-PCR. (Top) mRNAs from each specimen were isolated from the 8 people shown in Figure 1. RT-PCR was performed. Ethidium bromide-stained gel electrophoresis of *ZIP1* shows the relative degree of the amplifications. Lanes 1, 2, 3, and 4 are from white tissues, whereas lanes 5, 6, 7,and 8 are from the specimens from African Americans. (Middle) Amplifications of *hZIP2* from the total cellular mRNA isolated from the 6 neoplastic lesions. These specimens were randomized, and RT-PCR was performed for *hZIP2* on the 6 specimens shown in Figure 2. Lanes 1, 2, and 5 are from specimens from African Americans. Lanes 3, 4, and 5 are from specimens from white subjects. (Bottom) mRNAs isolated from the 8 specimens and tested for hZIP1 (top) were also tested for the presence and integrity of β-actin mRNA. Amplification of β-actin from the total cellular mRNA was successful, and there was no significant difference in the degree of amplification between the specimens isolated from white people and African Americans.

zinc transporters were not caused by relative degradation of mRNA signals in different specimens.

In 2 of 28 specimens from African Americans, we observed a significant upregulation of 1 of the 2 *hZIP* transporters, but not both. As shown in Figure 4, two prostate neoplastic lesions exhibited an overexpression of either *hZIP1 or hZIP2*, but not both. The inheritance of the overexpression of *hZIP1 or hZIP2* could have resulted from the interbreeding that occurred in past generations between the white and African American parents (or grandparents) of these people. This could have resulted in the correction or overcorrection of the genetic downregulation of the *hZIP1* or *hZIP2* transporters. These observations also point toward a promoter-mediated regulation of these 2 zinc transporters. This possibility is currently being evaluated in our laboratory.

To determine whether the relatively low expression of *hZIP1 and hZIP2* in African Americans is limited only to neoplastic areas, we examined the prostate tissues from normal, nonneoplastic tissues from healthy men of both races who died because of automobile accidents. As shown in Figure 5, there is a high degree of the expression of both *hZIP1* and *hZIP2* within the normal prostate tissues from 3 normal white males, whereas the expression in the prostates of 2 healthy African Americans was, at best, moderate.

DISCUSSION

African Americans have the highest prostate cancer incidence rate in the world.[13–18,25] At a global level, the rates of incidence are low in Asian and African men, low to moderate in white men, and high in African American men.[13,25] Using data collected between 1988 and 1992, Wingo et al[14] reported that African Americans have a 35% higher incidence rate and a 223% higher mortality rate from prostate cancer when compared with whites.

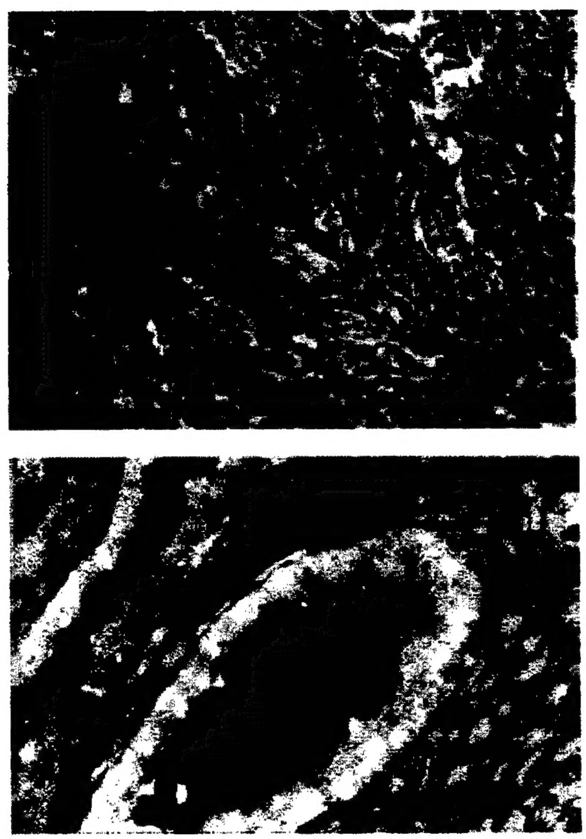
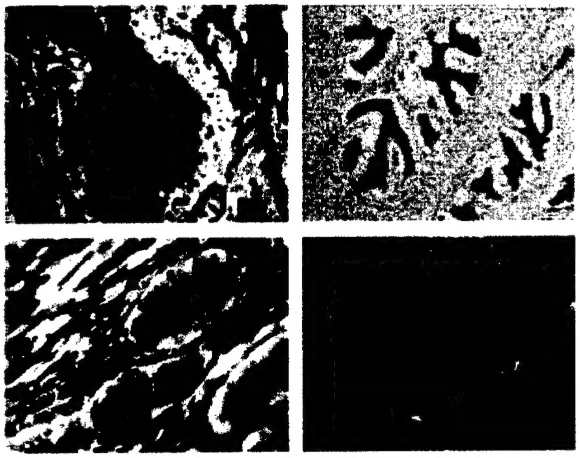


FIGURE 4. Representative photomicrographs from 2 neoplastic specimens from African American men. (Top) Upregulation of *hZIP1* (red) and relative downregulation of *hZIP2*. (Bottom) Upregulation of *hZIP2*, whereas *hZIP1* expression is almost absent.



ED5 **FIGURE 5.** Representative photomicrographs showing the relative expression of 2 zinc transporters, *hZIP1* and *hZIP2*, in the normal prostate glands by multiplex RT-in situ-PCR. The relative expression of *hZIP1 and hZIP2* in 2 normal prostate tissues from white men (left) and 2 age-matched African American men. Note that there is a high degree of expression of both *hZIP1* and *hZIP2* within the normal prostate tissues from 2 normal white men, whereas the expression in the prostates of 2 normal African American men would be at best scored as moderate.

The differences in the incidence and mortality between African Americans and whites are attributed to screening, environmental, and biologic factors.[16,17] When compared with white controls, black men present at a younger age with a higher grade and stage of the disease for their age, and with a greater delay in diagnosis.[18,19] Whether the pathogenesis of prostate cancer is different in African American men compared with white men remains unanswered. AU21 Whittemore et al[19,25] note that African American men appear to have a larger volume of "latent prostate cancer." These investigators believe that larger-volume latent carcinomas are those that progress to become clinically evident at a faster rate, suggesting that the events that account for racial differences in prostate cancer incidences may occur very early in cell transformation, and thus may be genetically controlled.

We have hypothesized that the major reason for the high incidence of prostate cancer in African Americans may be their inherent inability to absorb or transport normal amounts of zinc from the Northern American environment, in which there is a relatively low concentration of zinc in the diet. We have further hypothesized that zinc absorption and transport systems are genetically downregulated in some of the African populations. Such natural selection may occur because of the serious adverse effects on the nervous system caused by high zinc levels.[26–28] However, when African people entered North America, mostly during the slave trade, they may have encountered an environmental disadvantage because of their inherent downregulation of zinc transporters. On this continent, the zinc levels are relatively low, and the native populations (the American Indians) and other races who migrated from low zinc areas, ie, Europe and Asia, have a higher capacity to absorb zinc and are able to transport it to the other organs in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in white and nonwhite populations. The white population is unable to upregulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect themselves from the damaging solar UV light of wavelengths between 290 and 320 nm. Skin cancer is the most common type of cancer in the United States; more than 600,000 cases of skin cancer are reported each year in this country in the white population. Squamous and basal cell carcinomas are the most common tumors of sun-exposed skin areas in this group.[21] Just as white people carry an evolutionary disadvantage against the solar UV light outside their low UV light ancestral environment, the low absorption capacity of zinc has created a disadvantage in people of African descent when they migrate outside Africa. Long-term low serum concentration of zinc deprives the prostate gland of its essential source of a vital trace mineral ingredient, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the association of low levels of zinc with prostate neoplasia.[3–6] Zinc is an essential nutrient to all organisms because it is a required catalytic or structural cofactor for 100s of zinc-dependent enzymes and other proteins such as transcription factors. An example of effect of low serum levels of zinc can be seen in the animal model of *lethal milk* mouse mutant.[29] ZnT4 is deficient in the *lethal milk* mouse mutant, in AU22 which pups of any genotype suckled on homozygous *lethal milk* mothers die of zinc deficiency before weaning. The zinc level in the milk of homozygous *lethal milk* animals is approximately 50% that of normal animals, demonstrating that ZnT4 plays a crucial role in development.[29] Various reports suggest that regardless of race and geographic location, the etiology of certain prostate carcinomas may be linked to zinc transporters. Because the expression of zinc transporters also appears to be regulated by prolactin and testosterone, an age-related increase in the incidence rate of this malignancy may also be indirectly linked to the zinc uptake by the prostate gland.[8–11]

Members of the *ZIP* family are found in all cellular life forms, including archaebacteria, eubacteria, and eukaryotes.[10,30–34] AU23 There are currently approximately 85 members reported in the sequence databases. These fall into 4 subfamilies based on their amino acid similarities.[10] Most members are predicted to have 8 transmembrane

domains and share a predicted topology where the amino and carboxyl termini are extracytoplasmic. There are 12 known ZIP members in the human genome.[31] Three of the human proteins, *hZIP1*, *hZIP2*, and *hZIP3*, are very closely related to the fungal and plant proteins known to be zinc uptake transporters. *hZIP2* expression has been detected only in prostate[31] and uterine[32] epithelial cells, suggesting that this protein plays a very specialized tissue-specific function. On the other hand, hZIP1 is expressed in all 24 human tissues examined so far.[33] Gaither and Eide[10,11,31] have sequenced and characterized the *hZIP2* gene, a human zinc transporter identified by its similarity to zinc transporters recently characterized in fungi and plants. *hZIP2* is a member of the *ZIP* family of eukaryotic metal ion transporters that includes 2 other human genes, *hZIP1* and *hZIP3*, and the genes in mice and rats.[10,11]

The human genome contains at least 3 *ZIP* family members. The current hypothesis is that these genes encode zinc uptake transporters.[10,11] We can gain some insight into *ZIP* function in humans by considering the tissues in which these proteins are expressed. Repeated attempts by Gaither and Eide[11] to detect *hZIP2* mRNA on Northern blots of poly (A)+ RNAs derived from different human tissues and cultured cell lines failed to produce positive results. It appears that the *hZIP2* transporter gene is normally expressed at low levels and in specific cell types, and that a more sensitive detection method is required. We also attempted to quantitate *hZIP2* mRNA by Northern blots; however, several attempts were not productive. Therefore, we decided to use highly sensitive RT-in situ-PCR and SQ-RT-PCR methods. Gaither and Eide[10] isolated only 4 *hZIP2*-expressed sequence tag clones found only in prostate and uterine cDNA libraries. The observation that these particular tissues express *hZIP2* may be instructive in that cells of the prostate contain the highest zinc level of any soft tissue in the body. Any potential downregulation in this transporter may play a pivotal role in the pathogenesis of prostate cancer. Thus, it appears that the expression of *hZIP2* in prostate and uterine tissues may help meet their particular needs of zinc metabolism. In contrast, the low-affinity *hZIP1* and *hZIP3* have been cloned as expressed sequence tags from a large number of different tissues, indicating that these genes are widely expressed and may play general housekeeping roles.[10]

Therefore, observed zinc transporter expression may be associated with the great need for zinc involved in the normal processing of the prostate gland functions, a lack of which may have caused the molecular injury resulting in the development of prostate cancer.[7–11] Low serum levels of zinc have been associated with the increased incidence of prostate cancer.[7–12] Previously, Costello et al[8,9] have shown that *hZIP1* is expressed in PC-3 cells, and that a zinc update actively upregulated by testosterone and prolactin treatment. Furthermore, *hZIP1* expression was regulated by zinc availability. Therefore, when PC-3 cells were exposed to high zinc, *hZIP1* mRNA levels were downregulated. The molecular mechanisms by which low zinc levels contribute to the development of neoplasia are still obscure, and limited data are available. Costello et al[9] have shown that long-term cellular zinc deficiency leads to an increase in cell proliferation partly because of a reduction in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial apoptogenesis, indicating a physiologic effect of zinc in the regulation of prostate cell growth.

Thus, in prostate cancer, 2 themes emerge from the analyses of zinc transporter expression in vivo: (1) the downregulation of zinc transporters by either genetic inheritance (African descent) or through aging (related to the modulations in the testosterone/prolactin levels or gene expressions acquired with old age) leads to the low accumulation of zinc in the prostate tissues,[12–19] and (2) the loss of the unique capability to retain normal intracellular levels of zinc caused by either the increased export or low import of zinc may be an important factor in the development and progression of malignant prostate cells.[1–5,10,29–35] From our data, it appears that the lowest degree of the expression of zinc transporters, *hZIP1* and *hZIP1*, is localized in the areas that exhibit neoplastic lesions, and is less dominant in the areas that are healthy-appearing. Our observation that there are differences in the zinc transport in different racial groups has great significance for prevention. If a role of zinc transporters is clearly established, then a zinc supplementation could be helpful in at least some people. Understanding the molecular events in the pathogenesis of prostate cancer is critical to the evaluation of the natural history of prostate cancer in humans, especially in various racial groups.[34–38] □

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